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Garlon

Studies on the toxicity of garlon were summarized in an EPA document, "EPA No. 464-LUA. Garlon 3A Herbicide. Application for registration of new chemical formulation. Caswell No. 882I" (March 2, 1978). The communication was in response to a request for registration of Garlon 3A Herbicide, Dow Chemical Company, containing 44.4% (w/w) Trichlopyr (3,5,6-trichloro-2-pyridinyl-oxyacetic acid, as the triethylamine salt). Thereafter in the EPA summary of studies submitted by Dow, the nomenclature used for the herbicide was 3,5,6-trichloro-2-pyridyloxy acetic acid. The results summarized in the EPA document are presented in this report.

The adequacy or inadequacy of the individual tests cannot be evaluated because of the nature of the summary data provided. It will thus be impossible to compare without qualification the data available on garlon to that available on the other forest use pesticides for purposes of assessing relative risks.

Metabolism

No relevant data were provided for review.

Acute Effects

The acute toxicity of 3,5,6-trichloro-2-pyridyloxy acetic acid (technical grade) was reported to be:

Acute oral LD_{50}, rats, male and female	713 mg/kg
Acute oral LD_{50}, "cavies" (guinea pigs), male	310 (236-407) mg/kg
Acute oral LD_{50}, rabbits, male and female	550 (300-1,000) mg/kg
Acute dermal LD_{50}, rabbits	>2,000 mg/kg

From these results, the test substance was considered to be within Toxicity Category III for rats and Toxicity Category II for cavies and rabbits.

In the acute oral tests, single oral doses of 0.126-2.0 g/kg were given to five groups each containing five rats.

A primary eye irritation study in which three rabbits were dosed with 100 mg of test material indicated slight to moderate conjunctival redness lasting more than 7 days and very slight reversible corneal injury. The scoring system was not given.

A primary skin irritation test in rabbits was reported. Dry and wet applications of 1-2 g on intact skin (10 daily applications) and abraded skin (three daily applications) were made on the bellies of six rabbits. Slight redness, exfoliation, and swelling were reported. However, because of an "unusual

protocol," repeated applications, and absence of a scoring system, EPA noted that interpretation of the results was difficult.

In an acute dermal study, 2 g/kg of the test substance were applied to the unabraded skin of three albino rabbits and the abraded skin of another three albino rabbits. No adverse effects or mortalities were observed during the 2 weeks following the 24-hour exposure.

The acute toxicity of a herbicidal formulation (M3724) containing (Dowco 233) 3,5,6-trichloro-2-pyridyloxy acetic acid triethylamine salt (43.8%) and inerts (56.2%) was also summarized. The reported LD_{50}s were:

Acute oral LD_{50}, rats, female	2,140 (1,540-2,990) mg/kg
Acute oral LD_{50}, rats, male	2,830 mg/kg (no conf. limit)
Acute dermal LD_{50}, rabbits	>3,980 mg/kg
Acute inhalation LC_{50}, rats	>5.34 mg/liter/hour (3:20 dilution)

From these results, the test substance was considered to be within Toxicity Category III for rats.

In the acute oral tests, there were five animals per dose group, four dose groups for females and five dose groups for males. Female doses ranged from 500-3,980 mg/kg, while male doses ranged from 500-7,950 mg/kg. Most deaths occurred at 0.5-24 hours. Signs and symptoms recorded after the acute

oral doses included lethargy, narrowed eyes, and piloerection (for first 7 hours), and tremors and convulsions in two females.

A primary eye irritation test using 0.1 ml of this formulation resulted in severe conjunctival irritation, moderate iritis, and moderate to severe corneal injury in all six rabbits at 24 hours. These conditions persisted for at least 7 days in most animals in both washed (tap water) and unwashed eyes. Washing had no effect. Because the corneal opacity was not reversible within 7 days, the test substance was considered to be within Toxicity Category I.

In a primary skin irritation study, six rabbits given 0.5 ml of test material for 3 days on intact and abraded skin showed slight to moderate erythema, slight edema, and slight to moderate necrosis lasting at least 72 hours. The results of this study indicated that the test substance was within Toxicity Category II (based on necrosis at 72 hours). However, EPA noted that the repeated applications made interpretation of the study difficult.

In the acute inhalation study, 10 male and 10 female rats were exposed to "aerosols of a 3:20 aqueous dilution for 1 hour. Nominal aerosol concentration was calculated to be 5.34 mg/liter and mean aerosol particle size was 2.3 microns (99.9%<7.0 microns). No sign of toxicity, irritation, or mortality was observed in the 2 weeks following exposure.

Subchronic Effects

The subchronic toxicity of 3,5,6-trichloro-2-pyridyloxy acetic acid (Dowco 233) was evaluated in a 90-day feeding study in rats. A 14-day preliminary range-finding study showed decreased body weight gain at 300 and 200 mg/kg/day in both sexes and at 100 mg/kg/day in males only. "Mottled livers" were reported at necropsy, and the no-effect level was stated to be 30 mg/kg/day. In the 90-day study, Sprague-Dawley rats (minimum of 10 males and 10 females at each dose level) were fed 0, 3, 10, 30, and 100 mg/kg/day of 3,5,6-trichloro-2-pyridyloxy acetic acid. No deaths related to ingestion of the test material were reported. Body weights for males receiving 100 mg/kg/day were significantly lower than controls beginning at 13 days and continuing to the end of the study ($p<0.05$); food consumption for these same animals over the same period was "generally" lower. Hematology, urinalysis, and clinical chemistry data were reported as "essentially" negative. Gross necropsy and histopathology data presented no abnormalities related to the test material except for significantly decreased body weights and absolute liver weights and increased brain-to-body weight and kidney-to-body weight ratios in males receiving 100 mg/kg/day. The no-effect level was stated to be 30 mg/kg/day.

One nephroblastoma was found in a 100-mg/kg female rat that died on day 57. The same rat also had an enlarged spleen that showed evidence of extramedullary hematopoiesis. EPA did

not consider the occurrence of a single nephroblastoma in a single animal to be a cause for concern.

Acute and Subchronic Effects of a Possible Garlon Metabolite

A toxicity profile with no raw data was presented in summary form for the possible Garlon metabolite 3,5,6-trichloro-2-pyridinol. The acute oral LD_{50}s were reported as follows:

Acute oral LD_{50}, rats,	male	794 (709-889) mg/kg
	female	870 (758-1,009) mg/kg
Acute oral LD_{50}, mice,	male	380 (333-433) mg/kg
	female	415 (367-469) mg/kg

The test material, suspended in 0.5% hydroxypropyl methyl cellulose (Methocel), was administered to rats and mice. Groups of 10 male and 10 female Sprague-Dawley rats were given one of five doses in the range of 794-1,260 mg/kg (selection of these doses followed preliminary range-finding studies). Signs of acute poisoning observed in all rats were flaccid paralysis with dyspnea and slight hypersalivation within 5 minutes. These symptoms occurred with equal severity in rats from all doses. All rats that died did so 10 minutes-4 hours after administration of the test substance. Rats that died developed a rigidity of the whole body (like rigor mortis) within seconds after death. Survivors appeared normal within 24 hours. Gross necropsies were negative. From the results, the test substance was considered to be within Toxicity Category III.

Groups composed of 15 male and 15 female Swiss mice (Cox strain) received one of five doses of the test substance in the range of 354-891 mg/kg. (Selection of these doses followed preliminary range-finding studies.) Signs of acute poisoning observed in the mice were similar to those observed in the rats. These signs appeared at higher doses within 2 minutes, at lower doses within 5 minutes. In addition, exophthalmia was observed in a few mice. Males appeared to be more severely affected. All mice that died did so 2 minutes-2 hours after administration of the test substance. From the results, the test substance was considered to be within Toxicity Category II.

A 90-day feeding study of 3,5,6-trichloro-2-pyridinol with rats was conducted at dosage levels of 0, 1.0, 0.3, 0.1, 0.03, and 0.01%. Each dose was given to 10 rats of each sex. The no-effect level was 0.1%. At 1%, both males and females exhibited decreased growth rates and evidence of diuresis during the entire study. Decreased food consumption was noted for females. Increased organ/body weight ratios for kidney, spleen, testes, and brain were reported, and were possibly attributed to the decreased body weights. Increased liver-to-body weight ratios were reported to be dose-related. Dry, bloody noses were observed during the first month. At 0.3%, liver-to-body weight ratios were significantly increased in females, and diuresis was noted for both males and females during the entire

period. The summary listed a number of inadequacies for this study, including the insufficient presentation of the gross pathology.

Neurotoxicity

No relevant data were provided for review.

Biochemistry

No relevant data were provided for review.

Chronic Effects

No relevant data were provided for review.

Teratogenic Effects

The teratology study summarized in the EPA report is invalid. However, another teratology study was submitted by Dow and summarized without raw data in an EPA document dated April 2, 1979. The title of this communication was "EPA No. 464-LUA. Submission of additional toxicity data (teratology study) to support registration of Garlon 3A Herbicide. Caswell No. 8821."

In a preliminary range-finding study, 3,5,6-trichloro-2-pyridyloxy acetic acid was administered orally on days 6-15 of gestation to pregnant female rats (five at each dose) at 0, 25, 50, 100, 200, and 400 mg/kg/day. The vehicle was aqueous Methocel (0.5%). The five dams that received 400 mg/kg/day died after 3-5 doses, and one dam that received 200 mg/kg/day died on day 16. Gastric lesions were observed upon necropsy. Signs of severe "discomfort" were observed intermittently in

all treated dams after dosing on days 8-15 of gestation. Body weight gains for 200-mg/kg/day dams were lower than for controls. At 200 mg/kg/day, the number of implantations and viable fetuses (litter sizes) were markedly decreased, whereas the number of resorptions was not increased. All fetuses were reported to be "normal externally."

In the primary teratology study, 3,5,6-trichloro-2-pyridyloxy acetic acid was administered orally to bred females on days 6-15 of gestation at 0, 50, 100, and 200 mg/kg/day (25 rats at each dose). Dams were killed on day 20, and the fetuses were examined. Clinical signs of maternal toxicity were observed (excessive salivation, rough hair, excessive shedding, abdominal distress, and mild tremors). Body weight gain and food consumption were significantly lower at 100 and 200 mg/kg/day. A slight, statistically insignificant, increase in resorption rate at all dose levels was observed. Retarded ossification of skull bones was significantly increased in litters of dams receiving 200 mg/kg/day. No major developmental abnormalities were observed at 50 or 100 mg/kg/day. At 200 mg/kg/day, a total of 277 fetuses from 23 litters were examined. Multiple developmental defects were observed in two fetuses and generalized edema in a third, suggesting the possibility that high doses of 3,5,6-trichloro-2-pyridyloxy acetic acid may induce a low incidence of malformations in fetuses.

Reproduction Studies

A three-generation (one litter/generation) reproduction study with 3,5,6-trichloro-2-pyridyloxy acetic acid incorporated in the diets of Sprague-Dawley rats at 0, 3, 10, and 30 mg/kg/day was reported. Evaluation of "standard reproductive parameters" revealed no effects due to the test material at any of the doses tested. Daily observations, weekly body weights, and food consumption also were not affected. The no-effect level was determined to be greater than or equal to 30 mg/kg/day.

Mutagenicity

The results of an in vitro and a subchronic in vivo host-mediated assay for mutagenesis were reported. In the direct in vitro assays, Salmonella typhimurium TA1530 and G-46 were plated directly with disks containing 0.1 ml of saturated solution of test material (Dowco 233) and observed for revertants. Saccharomyces cerevisiae D-3 was treated with the test material for 4 hours at 30°C and then plated for determinations of surviving population and recombinant red sectors. The positive control in all cases was ethyl methanesulfonate. No significant increases in mutant or recombinant frequencies were found with the test material.

In the host-mediated assay, 3,5,6-trichloro-2-pyridyloxy acetic acid was administered orally to mice at 0.7, 7.0, and 70.0 mg/kg, both as a single dose and as multiple doses (five times at 24-hour intervals) followed by intraperitoneal injection of the indicator organisms (S. typhimurium strains or S. cerevisiae).

Animals were sacrificed 4 hours after injection, and peritoneal fluid was recovered, diluted, and plated, and revertants or recombinants were reported. The test material induced no significant increases in mutant or recombinant frequencies.

In vivo cytogenetic studies in male Sprague-Dawley rats (10-12 weeks old) were conducted after administration of single and multiple oral doses of 0.7, 7.0, and 70.0 mg/kg. In the multiple dose study, 3,5,6-trichloro-2-pyridyloxy acetic acid was administered at these doses daily for 5 days. A positive control group (triethylene melamine) and two negative control groups (corn oil and saline) were also utilized. In the single dose study, rats were sacrificed 6, 24, and 48 hours after administration, while in the multiple dose study, all animals were sacrificed 5 days after the last administration of the test substance. No cells with chromosomal aberrations were observed in the test groups or negative control groups, although they were observed in the positive control group. Mitotic indices were reported to be within normal limits.

A dominant lethal assay for mutagenesis was conducted using Sprague-Dawley CD strain rats. The test material was administered orally to male rats at 0.7, 7.0, and 70.0 mg/kg for 5 days. Triethylene melamine was included as a positive control, and corn oil and saline as negative controls. After treatments, each male was sequentially mated to two untreated females each week for 7 weeks. Females were sacrificed 14 days after mating and examined for eight parameters. A decrease

was noted in the number of pregnant females/number of mated females (fertility index) at the 7.0 and 70.0 mg/kg dosage levels during the first week only (the importance of this finding is uncertain). The following effects were noted over much of the 7-week testing period at the 7.0 and 70.0 mg/kg dosage levels: an increase in the average number of resorptions (dead implants) per pregnant female; increased proportions of females with one or more dead implantations and with two or more dead implantations; and increased ratios of dead implants/total implants. The above results indicated a weak positive effect produced by 3,5,6-trichloro-2-pyridyloxy acetic acid in this dominant lethal assay.

Human Studies

No relevant data were provided for review.

References

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY. 1978. Garlon 3A herbicide. Application for registration of new chemical formulation. Caswell No. 882I. EPA No. 464-LUA. March 2, 1978. From: Edwin R. Budd/Toxicology Branch/RD/OPP (WH-567). To: Robert Taylur, PM 25/RD/OPP (WH-567)

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY. 1979. Submission of additional toxicity data (teratology study) to support registration of Garlon 3A herbicide. Caswell No. 882I. EPA No. 464-LUA. April 2, 1979. From: Edwin R. Budd, Toxicology Branch, HED (TS-769). To: Richard Mountfort, Acting PM 25/RD (TS-767)